ImmunoTools *special* Award 2019

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Preclinical development of a next-generation CAR-T cell therapy for multiple myeloma

Background

Multiple myeloma (MM) is a malignant proliferation of plasma cells in the bone marrow that affects two people each day in Belgium (1, 2). Although therapeutic advances have led to improved outcomes, MM still remains incurable (3). Refractory disease and relapses are frequently observed due to drug resistance. Therefore, the therapeutic need in MM remains considerable. In the past decade, immunotherapy has become an important asset in the treatment of various cancers, including MM (4). One of the immunotherapeutic modalities that has attracted much recent attention, is chimeric antigen receptor (CAR)-T cell therapy. These CARs are composed of an antigen-binding part (often an antibody-derived single-chain variable fragment) and a signaling domain, linked together with a membrane-spanning region. Target recognition by the CAR triggers T-cell activation by mimicking TCR signaling and induces cytotoxicity towards antigen-expressing tumor cells. Early-phase CAR-T cell clinical trials have been initiated in MM, mainly targeted towards the myeloma surface molecule B-cell maturation antigen (BCMA). Results from these trials are promising, with high clinical responses rates including complete responses (Anguille et al. in preparation). Unfortunately, responses are usually temporary and relapses have been described due to loss of BCMA expression following CAR-T therapy (5). Therefore, a great deal of research attention is currently being paid at improving the efficacy of the CAR-T cells and at overcoming tumor escape. Because patient safety is of paramount importance, we employ mRNA electroporation of mRNA encoding a CAR as an elegant alternative to permanent viral modification. mRNA electroporation allows for highly efficient transgene expression without genomic integration. Meanwhile, the CAR is only transiently expressed due to degradation and dilution of the mRNA in the cytosol over time. This self-limiting nature of RNA CAR-T

cells is expected to minimize the frequency and the duration of serious adverse events, allowing this methodology to serve as a platform for rapid evaluation of novel CAR constructs.

Project description

We hypothesize that targeting multiple antigens will broaden the anti-tumor response and thus reduce the chance of immune escape. To this extent, we have generated three novel second generation CAR constructs, which are evaluated expression and functionality (**ImmunoTools**). Furthermore, it is becoming increasingly clear that modifications of the current CAR structures can further enhance CAR-T cell efficacy. Together with the Biotherapy Research Institute of Fudan University, Shanghai, China, we have found that the hinge domain plays an important and previously underrecognized role in CAR-T cell functioning. Here, we will further elaborate on this work by incorporating a novel hinge region in our CAR constructs, which will be benchmarked to the traditional hinge constructs in terms of induction of cytotoxicity, cytokine production and differentiation (**ImmunoTools**). Creation of a suppressive tumor micro-environment by the tumor cells, however, can induce exhaustion of the CAR-T cells with associated upregulation of immune checkpoints (6). *In situ* regulation of the expression of immune checkpoint molecules might be a more subtle (and thus less toxic) approach compared to systemic checkpoint inhibitors. Building on our expertise (7), we will investigate the knock down of immune checkpoints using silencing RNA.

References

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